PB86-208790

Early Life-Stage Toxicity Test Methods for Gulf Toadfish, 'Opsanus beta', and Results Using Chlorpyrifos

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

1986

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before compl

1. REPORT NO.	2.	PB86-208790
EPA/600/J-86/088		

4. TITLE AND SUBTITLE (Journal Version)
EARLY LIFE-STAGE TOXICITY TEST METHODS FOR GULF TOADFISH (OPSANUS BETA) AND RESULTS USING CHLORPYRIFOS

5. REPORT DATE 1986

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
D.J. Hansen, L.R. Goodman, G.M. Cripe, and S.F. Macauley

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
same as #12

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Research Laboratory - Gulf Breeze, FL
Office of Research and Development
U.S. Environmental Protection Agency
Gulf Breeze, FL 32561

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/4

15. SUPPLEMENTARY NOTES
Ecotoxicology and Environmental Safety 11:15-22 (1986)

16. ABSTRACT

Gulf toadfish (Opsanus beta) were continuously exposed as embryos, sac fry and juveniles to technical chlorpyrifos in two 49-day early life-stage toxicity tests. Survival was significantly (α = 0.05) reduced only in 150 µg/ℓ). However, toadfish exposed to chlorpyrifos concentrations from 3.7 to 150 µg/ℓ weighed significantly less than control fish: 9% lower in 3.7 µg/ℓ to 62% lower in 150 µg/ℓ. The 96-hour LC50 for juvenile fish was 520 µg/ℓ. Concentrations of chlorpyrifos in toadfish and bioconcentration factors increased with increasing exposure concentration, a condition not generally observed with other marine fishes and other test chemicals. These results demonstrated the procedures for, and the practicality of, early life-stage tests with this marine species. We recommend the use of the gulf toadfish for comparative toxicity testing and for evaluating the toxicity of substances in conjunction with ontogenetical, physiological and histological investigations of this considerably studied genus. We do not recommend it for routine effects testing.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	9
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA/600/J-86/088
journal Article

ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 11, 15–22 (1986)

Early Life-Stage Toxicity Test Methods for Gulf Toadfish (*Opsanus beta*) and Results Using Chlorpyrifos[1,2]

DAVID J. HANSEN,[3] LARRY R. GOODMAN, GERALDINE M. CRIPE, AND STEPHANIE F. MACAULEY

U.S. Environmental Protection Agency, Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561

Received July 15, 1985

Gulf toadfish (*Opsanus beta*) were continuously exposed as embryos, sac fry, and juveniles to technical chlorpyrifos in two 49-day early life-stage toxicity tests. Survival was significantly ($\alpha = 0.05$) reduced only in 150 μg/liter. However, toadfish exposed to chlorpyrifos concentrations from 3.7 to 150 μg/liter weighed significantly less than control fish: 9% lower in 3.7 μg/liter to 62% lower in 150 μg/liter. The 96-hr LC_{50} for juvenile fish was 520 μg/liter. Concentrations of chlorpyrifos in toadfish and bioconcentration factors increased with increasing exposure concentration, a condition not generally observed with other marine fishes and other test chemicals. These results demonstrated the procedures for, and the practicality of, early life-stage tests with this marine species. We recommend the use of the gulf toadfish for comparative toxicity testing and for evaluating the toxicity of substances in conjunction with ontogenetical, physiological, and histological investigations of this considerably studied genus. We do not recommend it for routine effects testing. © 1986 Academic Press, Inc.

INTRODUCTION

Early life-stage toxicity tests with fishes provide a continuous exposure to toxicant from the embryonic stage to approximately 3 or more weeks into the exogenous feeding stage (ASTM, 1985) and have been shown to estimate reasonably results from full life-cycle toxicity tests with freshwater and estuarine fishes (McKim, 1977; Macek and Sleight, 1977; Hansen, 1985). The data are useful for hazard assessment (Cairns *et al.*, 1978; Dickson *et al.*, 1979; Gentile and Schimmel, 1985) and are required for water quality criteria derivation (Stephan *et al.*, 1985). Although early life-stage toxicity tests are now possible with five species of estuarine fishes [sheepshead minnow (*Cyprinodon variegatus*), California grunion (*Leuresthes tenuis*), inland silverside (*Menidia beryllina*), Atlantic silverside (*M. menidia*), and tidewater silverside (*M. peninsulae*)] (Goodman *et al.*, 1983, 1985a,b; Hansen *et al.*, 1983), the fishes represent only two families, Atherinidae and Cyprinodontidae, and complete life-cycle tests have been conducted with only one species, the sheepshead minnow (Hansen, 1985). This limits the usefulness of data interpretations on the comparative chronic sensitivities to toxicants of families of estuarine fishes and relationships between early life-stage and full life-cycle tests.

[1] Gulf Breeze Contribution No. 549; Narragansett Contribution No. 743.

[2] Although the research described in this article has been funded wholly or in part by the United States Environmental Protection Agency, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

[3] Now at U.S. Environmental Protection Agency, Environmental Research Laboratory, South Ferry Road, Narragansett, R.I. 02882.

Gulf toadfish, *Opsanus beta* (family Batrachoididae), are bottom-dwelling fishes found from Cape Sable, Florida to Yucatan, Mexico and in the West Indies (Hoese and Moore, 1977). They occur from shallow estuarine areas to offshore hard bottoms at depths to at least 36 m (Walls, 1975). Springer and Woodburn (1960) collected Gulf toadfish 20.8 to 300 mm in length and at salinities from 3.2 to 35.0‰. Gulf toadfish are secretive, prefer recesses, and are found around oyster bars (Gunter, 1945), in burrows (Reid, 1954), under rocks, shells, and under or in debris, such as cans, tires, and wood. They spawn in recesses by attaching their adherent eggs to a hard surface and the male guards the "nest" (Breder, 1941). Measurements by Breder (1941) indicated that the diameter of attached eggs averaged 4.1 mm. We obtained embryos from April 15 to August 1 in collections from Pensacola Bay, Florida. Some of these "nests" contained several hundred embryos in monolayer clusters with multiple developmental stages present. For several days after hatching, the yolk-sac remains attached to the substrate by an attachment disk during which time the yolk is absorbed prior to the fish becoming free swimming. This has been described in detail (Gudger, 1910; Dovel, 1960) for the Atlantic toadfish (*O. tau*) which has been the subject of extensive reviews of research (Robinson, 1961; Robinson and Schwartz, 1965; Schwartz and Bright, 1982).

This paper presents methods for conducting early life-stage toxicity tests with gulf toadfish. Results from two tests with the insecticide chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl phosphorothioate); Dow Chemical Co., Lot No. 1-5503-2] provide data on chronic effects on survival and growth and information about test replicability.

MATERIALS AND METHODS

The acute toxicity of chlorpyrifos to toadfish was determined in a 96-hr static toxicity test with 24-hr renewals of test solutions. Duplicate 3-liter glass jars contained filtered seawater that ranged from 25 to 27°C temperature and 29 to 30‰ salinity. Eight toadfish that averaged 99 mg wet wt (range 73 to 116 mg) were tested in each duplicate. A seawater and solvent (triethylene glycol) control were maintained, along with chlorpyrifos concentrations of 0.65, 1.1, 1.8, 3.0, and 5.0 mg/liter. Chlorpyrifos in test solutions was measured just prior to renewal at 48 and 96 hr, and concentrations ranged from 50 to 60% of nominal in five samples from three treatments where partial kills were observed. Probit analysis was used to calculate the 96-hr LC_{50} and 95% confidence intervals (Finney, 1971).

Seawater used in each 49-day early life-stage toxicity test conducted sequentially was also pumped from Santa Rosa Sound, Florida, through a sand filter and a 20-μm polypropylene filter to an aerated reservoir where it was heated to approximately 25°C before use. A diluter similar to that described by Schimmel *et al.* (1975) cycled approximately every 0.25 hr, delivering 1 liter of exposure water to aquaria containing the seawater and solvent control and to five chlorpyrifos treatments of 12, 25, 50, 100, or 200 μg/liter in the first test and the solvent control and six chlorpyrifos treatments of 1.5, 3.0, 6.0, 12, 25, or 50 μg/liter in the second test. Water flowed from the diluter to seven 14.5 × 9 × 15.5-cm-high splitter boxes with two 7-mm ID self-starting siphons and from the splitters to fourteen 22 × 22 × 10-cm-high aquaria. Water depth in aquaria was varied from 2 to 7 cm by using a 12 mm OD glass self-starting siphon. Aquaria were partly immersed in a water bath that maintained test water at 26 ± 2°C.

Salinity ranged from 23.5 to 33.0‰ (average 29.5‰) for the first test and from 25.0 to 34.5‰ (average 29.4‰) for the second. Dissolved oxygen was measured weekly in each treatment and ranged from 4.1 to 6.4 mg/liter. The photoperiod was 12 hr light: 12 hr dark. Subsequent references to water concentration will be the average of chlorpyrifos concentrations measured weekly. At test termination survivors were analyzed for chlorpyrifos. Water and tissue analytical methods were those of Schimmel *et al.* (1983). The concentration of the solvent, triethylene glycol, was 40 μl/liter in the solvent control and in all chlorpyrifos treatments.

Gulf toadfish embryos attached to rocks or drain tiles were collected from the local estuary by divers, gently separated from the substrate after collection, and used for testing within 24 hr after collection. Embryos that were visibly developing were placed randomly into each of 28 incubation cups in groups of either three or four. This process was repeated until each cup contained 16 embryos in the first test and 15 embryos in the second test. Then the cups were randomly assigned, two to an aquarium. Incubation cups were glass petri dish bottoms (9 cm, ID) with 12-cm-high cylinders of 2-mm nylon mesh attached to the inside walls with silicone adhesive. Chemical analyses of embryos indicated that they contained no detectable chlorpyrifos (<0.015 μg/g), other organophosphate insecticides (<0.05 μg/g), chlorinated insecticides, or polychlorinated biphenyls (<0.002 to <0.10 μg/g for the 40 substances examined), except for embryos used in the first test, which contained 0.017 μg/g DDE.

Incubation cups were removed daily from aquaria to observe embryos and hatched fish for deaths and other signs of poisoning, such as abnormal embryonic development, activity, coloration or feeding. Because hatched fry contain significant yolk reserves (Martin and Drewry, 1978), they were not fed newly hatched *Artemia* until most of the yolk was resorbed, about 10 days after hatching. During the first 2 or 3 weeks, fry were fed two or three times daily at approximately 5000 *Artemia* (3200 to 6700) per cup per feeding. Thereafter, they were fed as frequently, but about four times that amount, average 19,200 per cup per feeding (range 6,300 to 44,300). They were not fed 24 hr before test termination. At termination of the experiments, fish were killed in hot water, towel drained, weighed individually, composited within exposure concentration, and frozen for subsequent chemical analysis.

Analysis of variance and Duncan's multiple range test were used to analyze weight and arcsine-transformed percentage survival data. The significance level was $\alpha = 0.05$.

RESULTS

Gulf toadfish exposed to chlorpyrifos in two early life-stage toxicity tests weighed significantly less than control fish; survival was a much less sensitive endpoint than growth (Tables 1 and 2). At test termination, surviving fish were smaller in all chlorpyrifos concentrations from 3.7 to 150 μg/liter than fish in the solvent control. The decrease in weight was concentration-dependent, ranging from 91% of solvent control weights in 3.7 μg/liter to 38% in 150 μg/liter.

Survival of sac fry and of juveniles following yolk resorption was significantly reduced (42%) only in the highest concentration tested, 150 μg/liter (Table 1). In contrast, the 96-hr LC_{50} was 520 μg/liter, (95% confidence limits 450 to 600 μg/liter). Overall survival of embryos and fry averaged 95.3% (range 83 to 100%) in the control and in those chlorpyrifos exposures where survival was not significantly reduced (≤93 μg/liter).

Development of sac fry was delayed, as indicated by the presence of the attachment disk. After sac fry in all other treatments had released the disk 25% (16 of 63) of the sac fry in 150 μg/liter retained their disk. Hyperactivity and hyperventilation were common in affected fish.

Chlorpyrifos was detected in toadfish that survived both experiments (Tables 1 and 2). Concentrations in whole fish increased from 0.14 to 770 μg/g with increase in exposure concentration. Bioconcentration factors (BCF, chlorpyrifos concentration in whole fish, wet weight, divided by average chlorpyrifos concentration measured in water) also increased from 100 to 5100 with increase in exposure concentration.

DISCUSSION

Early life-stage toxicity tests can be conducted with the Gulf toadfish. Embryos can be removed from nests in the estuary and placed in the laboratory where they will develop and hatch. Overall survival of embryos and fry in the 49-day tests was excellent: average survival was 95.3%. Brine shrimp nauplii (24- to 48-hr posthydration of cysts) apparently are an acceptable diet for post-sac fry toadfish; approximately 10 days after hatching at 26°C. Test duration provided a minimum of 4 weeks exposure of toadfish following yolk resorption.

TABLE 1

TEST NO. 1 SURVIVAL, AVERAGE WEIGHT, PERCENTAGE OF AVERAGE WEIGHT OF SOLVENT CONTROL FISH, RESIDUES, AND BIOCONCENTRATION FACTORS (BCF = CONCENTRATION MEASURED IN TISSUE ÷ AVERAGE MEASURED CONCENTRATION IN WATER) OF GULF TOADFISH (*OPSANUS BETA*) CONTINUOUSLY EXPOSED TO CHLORPYRIFOS FOR 49 DAYS AT 26 ± 2°C AND 24 TO 33‰ SALINITY

Exposure concentration (μg/liter)		Survival (%)			Weight			
Nominal	Average measured	Embryos	Fry	Combined	Average (mg)	% of control	Residue (μg/g)	BCF
Seawater control	ND[a,b]	100	100	100	222.4	105	0.017	—
Solvent control[c]	ND[a,d]	98	84	83	212.6	—	ND[a]	—
12	18 (5.5)	98	98	97	160.3[e]	75	12.0	670
25	21 (4.0)	94	100	94	165.5[e]	79	9.9	470
50	50.0 (9.9)	98	98	97	137.6[e]	65	45.0	900
100	93.0 (11.0)	92	91	84	107.4[e]	50	175.0	1900
200	150 (24.0)	98	43[e]	42[e]	80.8[e]	38	770.0	5100

Note. Standard deviations of measured water concentrations are in parentheses.
[a] ND = not detectable (<0.015 μg/liter in water, <0.010 μg/g in tissue).
[b] Three of the seven samples contained detectable chlorpyrifos, range 0.019 to 0.24 μg/liter.
[c] Contained 0.04 ml triethylene glycol/liter of seawater.
[d] Three of the seven samples contained detectable chlorpyrifos; range 0.018 to 0.038 μg/liter.
[e] Significantly different ($\alpha = 0.05$) from carrier-control response.

TABLE 2

TEST NO. 2 SURVIVAL, AVERAGE WEIGHT, PERCENTAGE OF AVERAGE WEIGHT OF CONTROL FISH, RESIDUES, AND BIOCONCENTRATION FACTORS (BCF = CONCENTRATION MEASURED IN TISSUE ÷ AVERAGE MEASURED CONCENTRATION IN WATER) OF GULF TOADFISH (*OPSANUS BETA*) CONTINUOUSLY EXPOSED TO CHLORPYRIFOS FOR 49 DAYS AT 26 ± 2°C AND 25 TO 34‰ SALINITY

Exposure concentration (μg/liter)		Survival (%)			Weight			
Nominal	Average measured	Embryos	Fry	Combined	Average (mg)	% of control	Residue (μg/g)	BCF
Solvent control[a]	0.11 (0.05)[b]	97	100	97	210.7	—	ND[c]	—
1.5	1.4 (0.50)	100	95	95	205.5	98	0.14	100
3.0	3.7 (0.51)	98	98	97	191.7[d]	91	0.95	260
6.0	8.2 (4.8)	98	100	98	170.0[d]	81	2.2	270
12.0	9.7 (1.4)	100	97	97	165.9[d]	79	4.7	480
25.0	24.0 (3.4)	100	100	100	133.5[d]	63	15.0	620
50.0	46.0 (5.8)	100	100	100	121.8[d]	58	30.0	650

Note. Standard deviations of measured water concentrations are in parentheses.

[a] Contained 0.04 ml triethylene glycol/liter of seawater.

[b] Chlorpyrifos was not detected in one of eight samples. Mean and standard deviation were calculated using the seven detectable samples.

[c] ND = not detectable (<0.015 μg/liter in water, <0.010 μg/g in tissue).

[d] Significantly different ($\alpha = 0.05$) from carrier-control response.

The relationship between the acute and chronic sensitivities of a species may be useful in hazard assessment and criteria development, particularly if this relationship is similar across species for a chemical (Cairns *et al.*, 1978; Stephan *et al.*, 1985). For the gulf toadfish, the geometric mean of the estimated lower and upper chronic values (highest concentration that caused no significant adverse effect and the lowest concentration that caused a statistically significant adverse effect) was 2.3 μg/liter. This chronic value divided into the 96-hr LC_{50} (520 μg/liter) resulted in an acute/chronic ratio of 226. Acute/chronic ratios from early life-stage tests with chlorpyrifos and four marine atherinids, California grunion, tidewater silversides, Atlantic silversides, and inland silversides, were similar, ranging from 2.4 to 6.5; (Goodman *et al.*, 1985a,b). These atherinids are more acutely sensitive to chlorpyrifos, LC_{50} 0.4 to 4.2 μg/liter (Borthwick *et al.*, 1985; Clark *et al.*, 1985; Schimmel *et al.*, 1983) than the sheepshead minnow, 96-hr LC_{50} 136 μg/liter (Schimmel *et al.*, 1983); the gulf toadfish, 96-hr LC_{50} 520 μg/liter (this study); and the freshwater fathead minnow, LC_{50} 120–140 μg/liter (Jarvinen and Tanner, 1982). Acute/chronic ratios from early life-stage tests for these three less acutely sensitive fishes were markedly higher: 62 for the sheepshead minnow (Cripe *et al.*, in press); 226 for the gulf toadfish (this study); and 37 and 62 for the fathead minnow (Jarvinen and Tanner, 1982). Chronic values from these early life-stage tests with seven species and chlorpyrifos were somewhat more similar: range 0.37 to 3.2. However, results from a partial life-cycle test with the fathead minnow (Jarvinen *et al.*, 1983) suggest that early life-stage tests may significantly underestimate the chronic toxicity of chlorpyrifos.

Increase in BCFs with increase in exposure concentrations in these tests with the gulf toadfish was not typical of similar tests with chlorpyrifos and other species and has not been generally observed with marine fishes exposed to other pesticides at our laboratory. Average BCFs for chlorpyrifos in other fishes we tested were 770 for the California grunion (Goodman *et al.*, 1985a) 220 for the inland silverside, 460 for the tidewater silverside, and <420 for the Atlantic silverside (Goodman *et al.*, 1985b). The BCFs for the sheepshead minnow generally increased with increased food availability and chlorpyrifos concentration (Cripe *et al.*, in press). Increased BCFs for both gulf toadfish and sheepshead minnows were associated with exposure concentrations that affected growth and survival. Possible explanations include (1) an increase in metabolic rate, as suggested by hyperventilation, hyperactivity and decreased growth of affected fish; (2) increased bioavailability caused by possible solvent-induced supersaturation of chlorpyrifos in treatments that exceeded water solubility [73 μg/liter (Schimmel *et al.*, 1983)]; and (3) decreased depuration rates, possibly caused by physiological dysfunction (Spacie and Hamelink, 1982).

Our results demonstrated the procedures required to conduct, and the practicality of, early life-stage tests with Gulf toadfish. This method allows an examination of the relative chronic sensitivities of marine fishes from three families and six species. The toadfish is not recreationally or commercially important and it is probably not particularly acutely sensitive to contaminants (Clark *et al.*, 1985). Also, the test requires resource-intensive field collection of embryos and a longer exposure duration than that for other marine fishes.

CONCLUSIONS

We recommend the early life-stage toxicity test with Gulf toadfish be used for comparative toxicological evaluations and for evaluations of chronic sensitivity of toadfish as a genus that has received considerable attention relative to its ontogeny, physiology, and histology. We do not recommend this species for routine effects assessments.

ACKNOWLEDGMENTS

We thank J. C. Moore for chemical analyses of exposure water and toadfish and C. H. Deans for assistance with statistical analyses. We are particularly indebted to members of the Gulf Breeze laboratory dive team, J. M. Patrick, Jr., Divemaster, for their tenacity in collection of toadfish embryos.

REFERENCES

ASTM (1985). *Proposed New Standard Guide for Conducting Fish Early Life-Stage Toxicity Tests.* Draft No. 9. American Society for Testing and Materials.

BORTHWICK, P. W., PATRICK, J. M., JR., AND MIDDAUGH, D. P. (1985). Comparative acute sensitivities of early life stages of atherinid fishes to chlorpyrifos and thiobencarb. *Arch. Environ. Contam. Toxicol.* **14,** 465–473.

BREDER, C. M., JR. (1941). On the reproduction of *Opsanus beta* Goode and Bean. *Zoologica* **26**(21), 229–232.

CAIRNS, J., JR., DICKSON, K. L., AND MAKI, A. W. (eds.) (1978). *Estimating the Hazard of Chemical Substances to Aquatic Life.* ASTM STP 657. American Society for Testing and Materials, Philadelphia, Pa.

CLARK, J. R., PATRICK, J. M., JR., MIDDAUGH, D. P., AND MOORE, J. C. (1985). Relative sensitivity of six estuarine fishes to carbophenothion, chlorpyrifos and fenvalerate. *Ecotoxicol. Environ. Saf.*, **10**, 382–390.

CRIPE, G. M., HANSEN, D. J., MACAULEY, S. F., AND FORESTER, J. (In press). Effects of diet quantity on sheepshead minnows (*Cyprinodon variegatus*) during early life-stage exposures to chlorpyrifos. In *Aquatic Toxicology and Hazard Asssessment: Ninth Symposium.* American Society for Testing and Materials, Philadelphia, Pa.

DICKSON, K. L., MAKI, A. W., AND CAIRNS, J., JR. (eds.) (1979). *Analyzing the Hazard Evaluation Process.* Water Quality Section, American Fisheries Society, Washington, D.C.

DOVEL, W. (1960). Larval development of the oyster toadfish, *Opsanus tau. Chesapeake Sci.* **1**(3–4), 187–195.

FINNEY, D. J. (1971). *Probit Analysis,* 3rd Ed. Cambridge Univ. Press, London.

GENTILE, J. H., AND SCHIMMEL, S. C. (1985). Strategies for utilizing laboratory toxicological information in regulatory decisions. In *Concepts in Marine Pollution Measurement* (H. H. White, ed.), pp. 57–80. Maryland Sea Grant Publication, Univ. of Maryland, College Park, Md.

GOODMAN, L. R., HANSEN, D. J., CRIPE, G. M., MIDDAUGH, D. P., AND MOORE, J. C. (1985a). A new early life-stage toxicity test using the California grunion (*Leuresthes tenuis*) and results with chlorpyrifos. *Ecotoxicol. Environ. Saf.* **10**, 12–21.

GOODMAN, L. R., HANSEN, D. J., MIDDAUGH, D. P., CRIPE, G. M., AND MOORE, J. C. (1985b). A method for early life-stage toxicity tests using three atherinid fishes and results with chlorpyrifos. In *Aquatic Toxicology and Hazard Assessment: Seventh Symposium,* ASTM STP 854 (R. D. Cardwell, R. Purdy, and R. C. Bahner, eds.), pp. 145–154. American Society for Testing and Materials, Philadelphia, Pa.

GOODMAN, L. R., MIDDAUGH, D. P., HANSEN, D. J., HIGDON, P. K., AND CRIPE, G. M. (1983). Early life-stage toxicity test with the tidewater silverside (*Menidia peninsulae*) and chlorine produced oxidants. *Environ. Toxicol. Chem.* **2**(3), 337–342.

GUDGER, E. W. (1910). Habits and life history of the toadfish (*Opsanus tau*). *Bull. U.S. Bur. Fish.* **28**(2), 1073–1109.

GUNTER, G. (1945). Studies on marine fishes of Texas. *Publ. Inst. Mar. Sci.* **1**(1), 1–190.

HANSEN, D. J. (1985). Utility of toxicity tests to measure effects of substances on marine organisms. In *Concepts in Marine Pollution Measurement.* (H. H. White, ed.), pp. 33–56. Maryland Sea Grant Publication, Univ. of Maryland, College Park, Md.

HANSEN, D. J., GOODMAN, L. R., MOORE, J. C., AND HIGDON, P. K. (1983). Effects of the synthetic pyrethroids AC 222,705, permethrin and fenvalerate on sheepshead minnows in early life-stage toxicity tests. *Environ. Toxicol. Chem.* **2**(2), 251–258.

HOESE, H. D., AND MOORE, R. H. (1977). *Fishes of the Gulf of Mexico, Texas, Louisiana, and Adjacent Waters.* Texas A&M Univ. Press, College Station, Tex.

JARVINEN, A. W., NORDLING, B. R., AND HENRY, M. E. (1983). Chronic toxicity of Dursban (chlorpyrifos) to the fathead minnow (*Pimephales promelas*) and the resultant acetycholinesterase inhibition. *Ecotoxicol. Environ. Saf.* **7**, 423–434.

JARVINEN, A. W., AND TANNER, D. K. (1982). Toxicity of selected controlled release and corresponding unformulated technical grade pesticides to the fathead minnow *Pimephales promelas. Environ. Pollut.* **27**, 179–195.

MACEK, K. J., AND SLEIGHT, B. H., III. (1977). Utility of tests with embryos and fry of fish in evaluating hazards associated with the chronic toxicity of chemicals to fishes. In *Aquatic Toxicology and Hazard Evaluation ASTM STP634* (F. L. Mayer and J. L. Hamelink, eds.), pp. 137–146. American Society of Testing and Materials, Philadelphia, Pa.

MARTIN, F. D., AND DREWRY, G. E. (1978). Development of fishes of the mid-Atlantic bight. An atlas of egg, larval and juvenile stages. In *Stromateidae through Ogcocephalidae,* Vol. 6. U.S. Fish and Wildlife Service, Biological Services Program, FWS/OBS-70/12, January, 1978.

MCKIM, J. M. (1977). Evaluation of tests with early life stages of fish for predicting long term toxicity. *J. Fish. Res. Board Canad.* **34**, 1148–1154.

REID, G. K., JR. (1954). An ecological study of the Gulf of Mexico fishes, in the vicinity of Cedar Key, Florida. *Bull. Mar. Sci.* **4**(1), 1–94.

ROBINSON, P. F. (1961). *A Bibliography of Papers Dealing with the Oyster Toadfish, Opsanus tau.* Chesapeake Biol. Lab., Solomons, Md. Contr. 183.

ROBINSON, P. F., AND SCHWARTZ, F. J. (1965). *A Revised Bibliography of Papers Dealing with the Oyster Toadfish, Opsanus tau.* Chesapeake Biol. Lab., Solomons, Md. Contr. 284.

SCHIMMEL, S. C., GARNAS, R. L., PATRICK, J. M., JR., AND MOORE, J. C. (1983). Acute toxicity, bioconcentration, and persistence of AC222,705, benthiocarb, chlorpyrifos, fenvalerate, methyl parathion, and permethrin in the estuarine environment. *J. Agric. Food Chem.* **31**(1), 104–113.

SCHIMMEL, S. C., PARRISH, P. R., HANSEN, D. J., PATRICK, J. M., JR., AND FORESTER, J. (1975). Endrin: Effects on several estuarine organisms. In *Proceedings, 28th Annual Conference of the Southeastern Association of Game and Fish Commissions, White Sulphur Springs, WV, Nov. 17–20, 1974.* pp. 187–194.

SCHWARTZ, F. J., AND BRIGHT, B. B. (1982). *A Bibliography of Papers Dealing with the Oyster Toadfish, Opsanus tau, 1965 through April 1982.* Spec. Publ. Inst. Mar. Sci., Univ. N.C.

SPACIE, A., AND HAMELINK, J. L. (1982). Alternative models for describing the bioconcentration of organics in fish. *Environ. Toxicol. Chem.* **1**(4), 309–320.

SPRINGER, V. G., AND WOODBURN, K. D. (1960). *An Ecological Study of the Fishes of the Tampa Bay Area.* Fla. Board Conserv. Prof. Papers Ser. No. 1.

STEPHAN, C. E., MOUNT, D. I., HANSEN, D. J., GENTILE, J. H., CHAPMAN, G. A., AND BRUNGS, W. A. (1985). *Guidelines for Deriving Numerical National Water Quality Criteria for Protection of Aquatic Organisms and Their Uses.* National Technical Information Service, Springfield, Va.

WALLS, J. G. (1975). *Fishes of the Northern Gulf of Mexico.* T.F.H. Publ., Neptune City, N.J.

Reprinted from Ecotoxicology and Environmental Safety **11**, 15–22 (1986)
Academic Press, Inc. Printed in U.S.A.